The Tale of the Telomere: Implications for Prevention and Treatment of Skin Cancers

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Life on Earth has evolved in an often hostile environment, leading organisms to develop multiple protective mechanisms. Central among these are systems for minimizing DNA damage and thus maintaining genomic integrity. Complex enzyme systems for detecting and repairing DNA base damage are present in prokaryotic bacteria and have been retained throughout evolution. Nucleotide excision repair (NER), e.g., is remarkably similar between bacterial and human cells, and mutation in any of the major NER genes can produce a usually fatal human disease, xeroderma pigmentosum (XP), with principal manifestations in the skin, the target of environmental ultraviolet (UV) irradiation. The critical importance of maintaining genomic integrity is underlined by two further phenomena. First, DNA repair capacity is induced following DNA damage, allowing organisms to devote even more energy to DNA repair when stimulated by environmental insult. This so-called SOS response has been extensively studied in bacteria and a functional ortholog was more recently well documented in mammalian cells as well. Second, in higher organisms, additional pathways have evolved to assure that under circumstances of irreparable DNA damage, cells eliminate themselves from the proliferative compartment and thus do not propagate mutations.

Work in our laboratory and others has provided insights into the mechanism by which cells in higher organisms, including human cells, use these evolutionarily perfected strategies for avoiding the most devastating consequence of unrepaired DNA damage: cancer. The following sections describe a recently elucidated key role for telomeres as well as evidence that this innate cancer avoidance mechanism may be harnessed for the prevention and treatment of skin malignancies.

Telomere Structure and Function

Eukaryotic chromosomes end in telomeres, tandem repeats of TTAGGG and its complement in all mammalian cells (Morin, 1989; Greider, 1996). In human cells, telomeres are initially 10–14 kb pairs in length (Harley *et al*, 1990) with a single-stranded 3′ overhang of approximately 100–400 bases comprising tandem repeats of TTAGGG (Stewart *et al*, 2003). Critical shortening or absence of telomeres causes cells to recognize their chromosome ends as double-strand breaks (Sharpless and DePinho, 2004). Such chromosomes then undergo fusion or otherwise manifest instability, confirming that one role of telomeres is to "cap" and protect chromosome ends (Blackburn, 2001). The "end replication problem," DNA polymerase's inability to replicate the final bases on each strand prior to cell division, causes telomeres to shorten with each round of cell division (Levy *et al*, 1992). Because telomeres do not encode genes or regulatory sequences, the buffer zone of TTAGGG repeats prevents loss of genetic information as cells divide. Also, because of this progressive shortening, after approximately 60 post-natal rounds of cell division, telomeres reach a critically short length (Harley *et al*, 1990), after which cells will no longer divide, regardless of mitogenic stimulation, a state termed proliferative senescence (Levy *et al*, 1992). Thus, a second function of telomeres is to act as a biologic clock, instructing cells either that they are young and proliferative or old and non-proliferative. Work from the laboratory of Titia de Lange has identified a third major role of telomeres, the initiation of DNA damage responses, as described below.

Telomeres are normally in a loop configuration with the double-stranded chromosome folded back on itself (Griffith *et al*, 1999) and the loop secured by insertion of the 3′ overhang into the proximal double-stranded DNA where it is held in place by binding proteins, particularly telomere repeat factor 2 (TRF2) (van Steensel *et al*, 1998). Transfection of cells with a dominant negative TRF2 construct ($TRF2^{DN}$) removes the binding protein and opens the telomere loop, exposing the 3′ overhang that is otherwise concealed (van Steensel *et al*, 1998). After linearization of the chromosome, the 3′ overhang is rapidly digested and the ATM (ataxia telanglectasia mutated) kinase, the protein mutated in the disease ataxia telangiectasia, is activated (Karlseder *et al*, 1999). ATM in turn phosphorylates (activates) the p53 tumor suppressor protein and transcription factor (Karlseder *et al*, 1999). Depending on the cell type, cells then undergo apoptosis (Karlseder *et al*, 1999) or enter replicative senescence (van Steensel *et al*, 1998). Both of these behaviors are believed to represent protective DNA damage respons-

Abbreviation: pTT, thymidine dinucleotide

es: apoptosis because it removes from the tissue cells with extensive and presumably unrepairable DNA damage and senescence because it prevents further proliferation of cells at risk for malignant transformation.

Interpretation of replicative senescence, in addition to apoptosis, as a cancer prevention mechanism arises not only from a philosophical appreciation that non-dividing cells cannot give rise to a malignancy (Campisi, 2001), a condition by definition in which cell growth is dysregulated and continues indefinitely, but also from recent observations that acute DNA damage or overexpression of certain oncogenes can give rise to the same senescent phenotype as does prolonged serial passage (Serrano *et al*, 1997; Zhu *et al*, 1998) and that apoptosis and senescence are both mediated by DNA damage response proteins such as p53 (Campisi, 2001). In any case, both apoptosis and senescence are well-documented responses of malignant cells to cancer therapy (Schmitt, 2003).

It is thus apparent that experimental telomere loop disruption and aging (serial cell division) share a common final pathway with acute DNA damage, such as double-strand breaks. All three responses involve activation of phosphatidyl inositol-3-like kinase(s) such as ATM, the ATM-related (ATR) kinase, or the DNA dependent-protein kinase (DNA-PK), which in turn activate p53 and other effector proteins (Yang *et al*, 2003). The final cellular response, apoptosis *versus* proliferative senescence, depends on the cell type and not on the character of the initial stimulus. These observations have led us to hypothesize that the common final pathway begins not with activation of the kinase but rather more proximally, with disruption of the telomere loop and exposure of the 3′ single-stranded overhang sequence, in mammalian cells repeats of TTAGGG.

DNA Damage Responses in Skin

In human skin, the most common medically consequential forms of DNA damage result from UV exposure. UV plays a major role in the great majority of the more than 1.3 million basal and squamous cell carcinomas diagnosed each year in the US (ACS, 2004), and is implicated in more than two-thirds of all melanomas (Gilchrest *et al*, 1999). In addition to ample and/or intense intermittent sun exposure, major risk factors for skin cancer include fair complexion, poor tanning ability, and tendency to freckle, all indicative of vulnerability to UV insult (Fitzpatrick and Ortonne, 2003).

It has long been recognized that UV exposure directly damages DNA (Setlow and Carrier, 1966) and also stimulates increased melanogenesis, so-called tanning (Fitzpatrick and Ortonne, 2003), in skin that is genetically capable of this response (Fitzpatrick phototypes II–VI). Tanning is also well documented to protect against acute and chronic consequences of future UV exposures, including photocarcinogenesis (Fitzpatrick and Ortonne, 2003). Interestingly, in intact human skin, the action spectrum for production of UV-induced DNA damage, specifically for cyclobutane pyrimidine dimers (CPD), the most common DNA photoproduct, is identical to the action spectrum for tanning, peaking broadly at 300 nm, and then falling off by four to five orders of magnitude at longer UV wavelengths (Parrish *et al*, 1982; Freeman *et al*, 1989). This suggests a cause and effect relationship between formation of such photoproducts and the increased melanogenesis (tanning) observed over the subsequent 3–5 d (Gilchrest and Eller, 1999). Furthermore, chemical agents and restriction enzymes known to act exclusively by damaging DNA within cells also stimulate melanogenesis (Eller *et al*, 1996), strongly supporting the hypothesis that DNA damage itself plays a large role in triggering the tanning response after UV exposure. Finally, work by our laboratory and others demonstrated that the rate-limiting enzyme tyrosinase is a p53-regulated gene product (Nylander *et al*, 2000; Khlgatian *et al*, 2002) and that UV-induced melanogenesis is greatly reduced in p53 knockout animals (Gilchrest, 2004), further supporting an evolutionary role of tanning as a DNA damage response.

Tanning is thus a major photoprotective response of human skin to UV irradiation and is capable of decreasing DNA damage from future exposures by absorbing UV photons (Kollias *et al*, 1996). Also, in the human skin, melanin is arranged in a supranuclear cap that maximizes this DNA-protective effect (Kobayashi *et al*, 1998; Byers *et al*, 2003). The major form of DNA protection throughout evolution, however, has been enzymatic repair of the damaged DNA itself. Indeed, prokaryotic bacteria have not only an elaborate basal mechanism of DNA repair but also an inducible component of repair termed the SOS response (Radman, 1974, 1975). Specifically, UV irradiation leads to the generation of single-stranded DNA fragments that contain photoproducts, and this DNA forms a complex with the Rec A protein that then cleaves a transcription repressor, increasing the synthesis of approximately 20 bacterial genes that encode DNA repair and cell survival proteins (Walker, 1984). DNA damage thus induces protective responses that render bacteria more resistant to subsequent damage of the same type and hence more likely to survive in the injurious environment.

Experiments suggesting a eukaryotic SOS-like response have been reported sporadically since the 1970s. Using an experimental design similar to that used to demonstrate that prior UV irradiation of bacteria enhances their ability to repair UV-irradiated bacteriophage, human fibroblasts UV-irradiated 4 d prior to infection with UV-irradiated herpes virus were shown to support viral growth approximately twice as well as unirradiated control fibroblasts (Lytle *et al*, 1976). As in the earlier bacterial experiments, this implied that the host cells had enhanced DNA repair capacity following UV irradiation. Others later confirmed these findings in monkey kidney cells, using SV40 virus (Taylor *et al*, 1982). Moreover, the enhanced viral DNA repair by irradiated monkey cells was not associated with an increased mutation frequency, as had been observed in bacteria. Subsequently, human fibroblasts subjected either to ionizing radiation or UV irradiation were similarly demonstrated to have enhanced repair of virus DNA that had been damaged by the same insult prior to infection (Jeeves and Rainbow, 1983, 1986), and that fibroblasts from patients with ataxia telangiectasia, known to be hypersensitive to ionizing radiation, did not demonstrate this enhanced viral repair (Jeeves and Rainbow, 1986). Other investigators subsequently showed that human cells lacking p53 or expressing a dominant negative p53 also do not manifest the inducible DNA

repair response (Rainbow *et al*, 1995; McKay and Rainbow, 1996; McKay *et al*, 1997), consistent with the known activation of p53 by ATM and its central role in NER (Banin *et al*, 1998). Moreover, very recent data demonstrating activation of ATM by single-stranded DNA (Lee and Paull, 2005) were interpreted by the investigators as "consistent with the central role of single-stranded DNA as an evolutionary conserved signal for DNA damage." Thus, there is considerable support for the concept that mammalian cells have inducible protective DNA damage responses that are functionally analogous and perhaps even loosely analogous in their molecular mechanism to the bacterial SOS response, despite obvious discrepancies between the eukaryotic and prokaryotic systems.

Mimicking Telomere Disruption and Exposure of the 3′ Overhang

Wishing to test the hypothesis that DNA damage is a major stimulus for UV-induced tanning and unable to obtain a concentrated preparation of CPD for laboratory use, we asked whether exposure of cultured pigment cells or intact skin to thymidine dinucleotide (pTT), the obligatory substrate for the thymine dimers that account for approximately 75% of all UV-induced DNA damage (Setlow and Carrier, 1966; Matsunaga *et al*, 1991), might increase melanogenesis in the absence of UV exposure. Both cultured human melanocytes and intact guinea-pig skin responded to pTT treatment with dramatic increases in melanin production, precisely mimicking UV-induced tanning clinically and histologically (Eller *et al*, 1994). The tanning was attributable to increased mRNA and protein expression of tyrosinase, the rate-limiting enzyme in melanogenesis. Subsequent studies demonstrated that the pTT-induced tan in guinea-pig skin was highly photoprotective and thus reproduced the functional as well as clinical and histologic aspects of UV-induced tanning (Gilchrest and Eller, 1999).

We then asked what signaling pathways were activated by pTT. Initial experiments focused on p53, the Guardian of the Genome (Lane, 1992), known to mediate many DNA damage responses. pTT-treated human keratinocytes and fibroblasts, transfected with a non-replicating UV-damaged chorampheniсol acetyl transferase (CAT) reporter vector that had previously been damaged by UV irradiation, gave rise 24 h later to more than twice the CAT activity (a measure of vector repair) than did diluent-treated control cultures (Eller *et al*, 1997). pTT-treated fibroblasts showed a 2–3-fold increase in p53 protein levels and increased p53 activity as detected in an electrophoretic mobility shift assay in which p53 activation is assessed by binding to its consensus sequence in DNA. pTT treatment of human fibroblasts was subsequently shown to similarly increase the protein levels of p53, as well as several p53-regulated DNA repair enzymes over 3–5 d (Goukassian *et al*, 1999). Moreover, pTT treatment of older adult cells increased the levels of these proteins several fold, to the basal levels observed in newborn fibroblasts (Goukassian *et al*, 2002).

Functional consequences of p53 induction and activation could also be observed following pTT treatment, in that survival and clonogenic capacity of pre-treated UV-irradiated cells and rate of repair of CPD all increased appreciably (Eller *et al*, 1997). In subsequent experiments, the same CAT vector, treated with benzo[*a*]pyrene to induce DNA adducts rather than UV irradiated to induce CPDs, was used and vector expression in pTT-pretreated cells was again shown to be twice that in diluent-pre-treated controls (Maeda *et al*, 1999). To place these results in perspective, this CAT vector host cell reactivation assay has also been used to quantify the impact of DNA repair rate on cancer risk in large populations (Wei *et al*, 1993). Cells derived from young adult patients with basal cell carcinoma *versus* age-matched controls demonstrated a 5%–8% decrease in ability to repair the vector, and old *versus* young donor cells demonstrated a 15% decrease, differences in both cases assumed to be causally related to their increased cancer risk (Wei *et al*, 1993). In comparison, the pTT-inducible increases were 100%–200% of baseline rates (Eller *et al*, 1997; Maeda *et al*, 1999).

The effect of pTT pretreatment on DNA damage repair rates was also documented in intact guinea-pig skin by measuring the rate of removal of photoproducts following UV irradiation (Gilchrest and Eller, 1999). In bacteria, however, the increased rate of DNA repair during the SOS response is accompanied by increased mutation rate (Walker, 1984). Although this can be viewed as evolutionarily beneficial to bacteria, promoting environmental adaptation, mutations in higher organisms such as humans pose unwanted risks to the individual, ranging from compromised tissue function to carcinogenesis. The low-fidelity umuC and umuD bacterial repair enzymes induced during the SOS response (Walker, 1984) have no known human homologs, but it was nevertheless critical to determine whether the pTT-induced enhanced repair capacity increases the mutation rate following DNA damage. Three different host cell reactivation assays using murine cells, intact murine skin, and human fibroblasts demonstrated a substantial reduction in mutation rate in pTT-pre-treated cells (Hadshiew *et al*, 1999; Khlgatian *et al*, 1999; Ruenger *et al*, 2002). These results are consistent with the generally inverse relationship between DNA repair rate and mutation rate (Moriwaki *et al*, 1996) and the intuitive notion that an evolutionarily conserved inducible DNA repair capacity would increase the probability of maintaining an intact genome in mammalian cells repeatedly exposed to environmental mutagens (Fig 1).

Having established that pTT induces photoprotective tanning and increases DNA repair capacity at least in part via the p53 signaling pathway, we asked whether other oligonucleotides might also be effective in stimulating these potentially therapeutic responses. We found that several other, but certainly not all, oligonucleotides have effects similar to pTT, often at far lower molar equivalent doses, and that activity requires nuclear uptake, a phenomenon that appears to depend on the presence of a 5′ phosphate group (Hadshiew *et al*, 2001), as reported for cellular uptake of other oligonucleotides (Noonberg *et al*, 1993). Given the very interesting consequences of experimental loop disruption then recently reported in the literature (van Steensel *et al*, 1998; Karlseder *et al*, 1999) and their substantial overlap with the observed pTT effects, we asked whether homology to the telomere 3′ overhang might be the common feature of active oligonucleotides

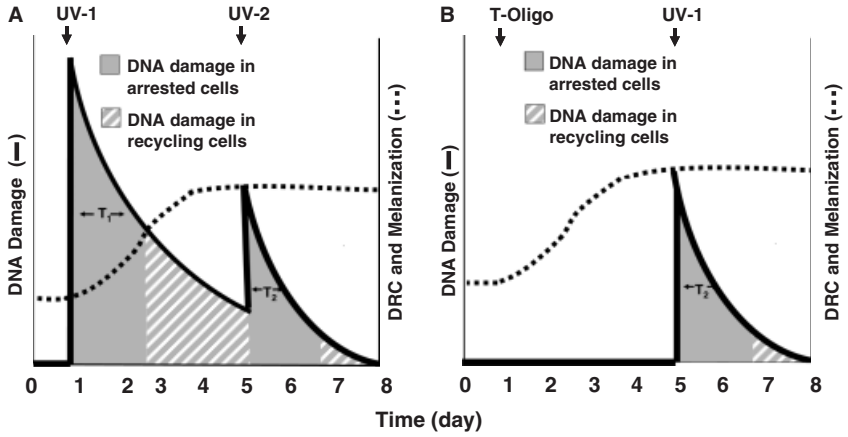

Figure 1
Consequences of the SOS response in human skin. (*A*) Under basal conditions, cells in the human skin have a relatively low DNA repair capacity (DRC) and melanin content. An initial UV exposure (UV-1) results in substantial immediate DNA damage (*solid line*). DNA repair, as measured by removal of DNA photoproducts, has been shown to occur exponentially, with the time for removal of 50% of initial photoproducts (T_1) determined to be approximately 24 h for thymine dimers and 6 h for (6–4) pyrimidine pyrimidone photoproducts following a physiologic UV exposure *in vivo* or *in vitro*. Over approximately 1–3 d following the UV exposure, however, DNA repair capacity increases up to 2–3-fold and over 3–5 d melanin content increases similarly, leading to a visible tan. If a second equal UV exposure (UV-2) then occurs, much of the incident radiation is absorbed by epidermal melanin, leading to the formation of fewer DNA photoproducts, and this lesser initial DNA damage is repaired more rapidly, with the time for repair of 50% of the damage (T_2) approximately half that following the initial exposure, as determined experimentally, due to enhanced DRC. Also, after each UV exposure, there is a concentration-dependent p53-mediated cell cycle arrest of approximately 1–2 d during which DNA repair occurs without risk of mutation. Following the second UV exposure, however, p53 induction and activation are greater and the period of cell cycle arrest is proportionately longer, providing the cell more protected time during which DNA repair can occur without risk of mutation. Once epidermal cells resume cycling, any remaining unrepaired damaged poses a risk of introducing a mutation into the newly synthesized DNA strand. The risk of mutation is indicated by the hatched areas under the DNA damage curves following the UV-1 *versus* UV-2 exposures and is far less in cells in which the SOS response has been induced. Eventually, in the absence of further UV exposures, DRC and melanin content return to baseline and any subsequent exposure resembles UV-1. But, additional exposures during the period encompassed by the SOS response (assumed to be 1–2 wk or longer, depending on the inciting UV exposure, DRC, and melanin content) are maintained or further induced and each exposure is handled like UV-2. Hence, the existence of the SOS response implies that the pattern of UV exposures, infrequent large exposures *versus* frequent smaller exposures, in addition to the total cumulative UV dose, determine the degree of damage in surviving cells. (*B*) pTT or other telomere homolog oligonucleotide (T-oligo) increases DRC and melanin content in cells over the same time course as a UV exposure or other acute DNA damage, but in the absence of DNA damage. Hence, a UV exposure several days after T-oligo treatment is handled like a second UV exposure in the panel A scenario, with less initial DNA damage and a far lower risk of mutation due to residual DNA damage in cells that have resumed cycling after the initial period of cell cycle arrest. Modified from Gilchrest and Eller (2001).

in our assay systems. This proved to be the case. All active oligonucleotides showed greater than 50% homology with the TTAGGG tandem repeat sequence, whereas inactive oligonucleotides failed to meet this criterion. In general, longer sequences (up to at least 20 nucleotides) were more effective than shorter sequences, and the presence of cytosine (C) residues specifically reduced activity (Hadshiew *et al*, 2001; Eller *et al*, 2002). Relative activity among test sequences was the same in all assay systems and did not vary with the biologic endpoint used. The shortest effective sequence, pTT, was noted to represent 100% of one-third of the tandem repeat sequence (Eller *et al*, 2002).

Our observation that telomere homolog oligonucleotides, which we subsequently termed T-oligos (Eller *et al*, 2003), specifically activate DNA damage responses is consistent with the hypothesized common final pathway for experimental telomere loop disruption, cellular senescence at the time of critical telomere shortening, and acute DNA damage responses (Fig 2*A*). Exposure of the otherwise concealed 3′ overhang was known to occur following telomere loop disruption by $TRF2^{DN}$ (Karlseder *et al*, 1999), and critical telomere shortening might also plausibly lead to loop disruption as a consequence of stochastic instability of a tighter loop (Li *et al*, 2003). Also, recent work has strongly suggested that, as in experimental loop disruption, loss of the overhang accompanies proliferative senescence (Stewart *et al*, 2002), consistent with its exposure and digestion. Although another technique has instead failed to show overall reduction in telomere overhang length in late-passage senescent cells, the investigators note that loss of the overhang on a minority of chromosome strands would not be detected but might still be sufficient to trigger cell senescence (Chai *et al*, 2005).

Finally, several groups have demonstrated that telomeres are preferentially targeted at times of acute DNA damage, whether due, e.g., to UV exposure (Oikawa *et al*, 2001), oxidative stress (Oikawa and Kawanishi, 1999; Kawanishi and Oikawa, 2004), or exposure to DNA-damaging chemicals (Kang *et al*, 2004). In this context, it is of interest to note that the TTAGGG sequence contains an abundance of preferred targets for these common DNA-damaging agents: one-third of the sequence is TT, a preferred UV target (Setlow and Carrier, 1966), and half of the sequence consists of G residues, the preferred target for reactive oxygen species and chemical carcinogens (Cheng *et al*, 1992). It is thus plausible that preferential damage to telomeres at the time of overall genomic damage might result in telomere disruption, as a consequence either of introducing photoproducts, chemical adducts, or the like, or as a consequence of attempted repair of these lesions. Thus, all three conditions might be expected to expose the TTAGGG repeat sequence, which interestingly does not otherwise appear to occur twice consecutively elsewhere in the human genome (NCBI Human Genome BLAST search [http:www.ncbi.nlm.nih.gov/genome], accessed in 2004), allowing it to interact with a sensor protein or protein complex, which in turn activates of the ATM, ATR, and/or DNA PK kinases with subsequent DNA damage signaling (Fig 2*B*).

Whether or not the hypothesized mechanism is responsible, we have documented extensive parallels between T-oligo treatment and experimental telomere loop disruption, as well as between T-oligo treatment and DNA damage responses following UV irradiation. These include not only tanning and cell cycle arrest but also senescence (Li *et al*, 2003; Gilchrest, 2004; Kosmadaki and Gilchrest, 2004) and apoptosis (Eller *et al*, 2002).

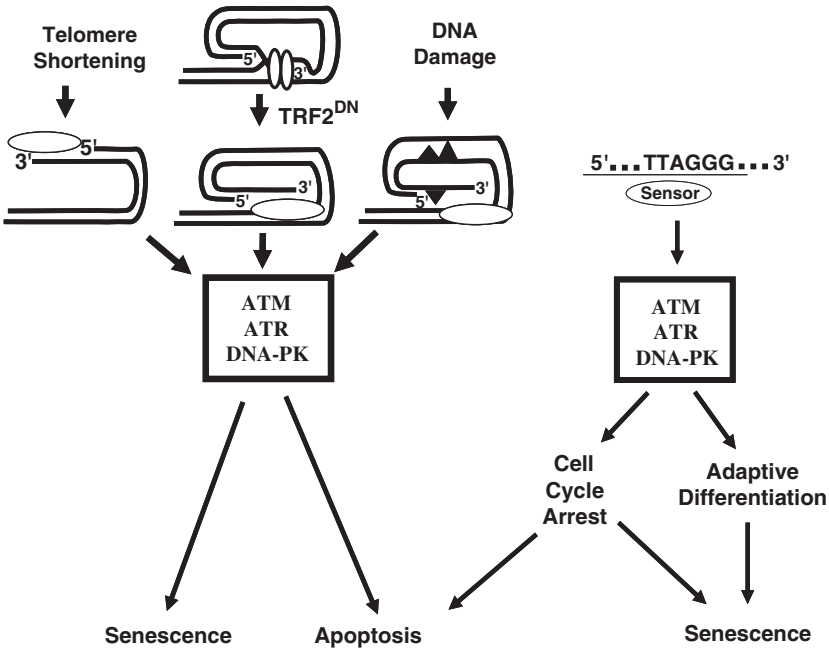

Figure 2
Hypothesized DNA damage signaling pathway following telomere loop disruption and exposure to the 3′ overhang sequence. (*A*) Just as experimental telomere loop disruption by dominant negative telomere repeat factor 2 (TRF2) construct exposes the telomere 3′ overhang sequence, we hypothesize that both serial cell division with consequent critical telomere shortening and acute DNA damage, such as UV irradiation with introduction of photoproducts, distort the telomere loop and expose the TTAGGG repeat sequence. We further hypothesize that in all three situations, the overhang sequence is recognized by a nuclear sensor protein or protein complex (open oval) that then initiates signaling through the ATM, ATM-related (ATR), and/or DNA dependent-protein kinase (DNA-PK) kinases to activate p53 and other effector proteins. Such intense or prolonged signaling results in senescence or apoptosis, depending on cell type. (*B*) We have observed that treatment of mammalian cells with oligonucleotides having substantial or complete homology to the telomere overhang sequence also initiates signaling through the ATM, ATR, and/or DNA-PK kinases, presumably because these T-oligos, known to enter the nucleus, are recognized by the same sensor protein. T-oligo treatment at low dose and/or short duration results in reversible cell cycle arrest, often with evidence of adaptive differentiation, such as enhanced melanogenesis in pigment cells, whereas higher doses or longer duration of therapy may push cells to the same cell-type-specific biologic endpoints of apoptosis or senescence as observed after serial cell passage, acute DNA damage, or experimental telomere loop disruption. Modified from Gilchrest (2004).

Potential Clinical Applications of T-Oligos

Enhancing DNA repair after UV irradiation while reducing the mutation rate would predict that T-oligo therapy might reduce photocarcinogenesis. To examine this possibility, we used the widely studied SKH1 hairless mouse engineered to express a lacZ mutation reporter transgene and a chronic UV irradiation protocol known to produce squamous cell carcinomas within 6 mo (Goukassian *et al*, 2004). Mice with both wild-type DNA repair capacity and mice heterozygous for deletion of the XPC repair enzyme were used to model the broad range of the DNA repair capacity observed in the human population. pTT or diluent alone was applied daily to the back for the first 5 d of each month, and then the mice were UV irradiated 5 d per wk for the remaining 3 wk of each month, for a total of 6 mo. The UV-irradiated mice progressively developed skin tumors as expected, and at the end of 6 mo only 12% of the control vehicle-treated mice were tumor free. Seventy-eight percent of the intermittently pTT-treated mice, however, were tumor free. Histologic analysis revealed both actinic keratosis-like lesions and invasive squamous cell carcinomas, with each tumor type being more prevalent in the vehicle-treated than in the pTT-treated mice. There was no evidence of pTT toxicity in either the irradiated mice or in sham-irradiated controls. Assessment of the lac-Z/pUR288 reporter plasmid showed a decreased mutation rate in pTT-treated *versus* control mice after either a single UV exposure or after 6 mo of intermittent irradiation, confirming the previous *in vitro* data (see above). These data suggest that topical T-oligo therapy might be a valuable adjunct to sunscreen use and sun avoidance in patients at high risk of photocarcinogenesis.

Our initial work with T-oligos fully homologous to the 3′ overhang demonstrated apoptosis in multiple established cell lines (Eller *et al*, 2002), including melanoma cells. Because apoptosis is a major mechanism by which chemotherapeutic agents reduce tumor burden, and resistance to apoptosis is a well-documented mechanism of treatment resistance (Schmitt, 2003), we asked whether T-oligos might also have a role in treating established malignancies. As a first experimental model in which to determine whether T-oligos can serve as an effective cancer therapy, we selected melanoma, the most fatal of skin cancers and a malignant cell type characterized by resistance to conventional cancer therapy once metastasis has occurred. In preliminary experiments, several human melanoma cell lines were examined and found to readily undergo apoptosis within 72–96 h of exposure to an 11-base T-oligo (pGTTAGGGTTAG). For further study, we selected the aggressive MM-AN line (Puri *et al*, 2004) known to lack p53 and $p16^{INK4a}$ expression, widely used in mouse models of melanoma and considered to be predictive of clinical responses (Paine-Murrieta *et al*, 1997; Jansen *et al*, 1998). T-oligos were added once to culture medium at time 0, and the cells were examined daily for 4 d. By 96 h, two-thirds of the MM-AN cells were undergoing apoptosis, mediated at least in part by the p53 homolog p73 (Eller *et al*, 2002). Interestingly, normal human melanocytes responded to T-oligo only with a transient cell cycle arrest and did not undergo apoptosis (Puri *et al*, 2004), suggesting that T-oligo-induced apoptosis preferentially affects malignant cells. Apoptosis of the MM-AN cells was explained in part by marked downregulation of livin/ML-IAP, a member of the inhibitor of apoptosis family of proteins known to be expressed normally during embryogenesis and inappropriately re-expressed in many melanomas, thereby contributing to their resistance to chemotherapy-induced apoptosis (Vucic *et al*, 2000; Kasof and Gomes, 2001). T-oligo-treated MM-AN cells also showed upregulation of the melanogenic proteins tyrosinase and TRP1, as well as the other differentiation markers gp100 and MART1, most

prominently after 72–96 h when many of the cells had already undergone apoptosis. To determine whether these effects could be seen *in vivo*, the immunocompromised SCID mouse model was used. T-oligo dramatically reduced tumor burden when MM-AN cells were treated either prior to tail vein injection, in a protocol that yielded numerous metastases in control animals, or following intralesional or intraperitoneal injection in animals with MM-AN cells implanted either subcutaneously or intraperitoneally (Puri *et al*, 2004). Small residual tumors in the T-oligo-treated animals contained cells undergoing apoptosis and/or showing evidence of differentiation by immunostaining, whereas control tumors did not. As in the cancer prevention studies, there was no evidence of T-oligo toxicity following either systemic or local injection daily for up to 26 d. These data suggest that T-oligos may provide a safe and effective novel means of treating advanced melanoma.

Summary and Conclusions

Work in many laboratories over the past decade has established a central role for the telomere in maintaining genomic integrity. Available data may be interpreted to indicate that telomere disruption, whether due to acute DNA damage or progressive telomere shortening, is the initial event that triggers multiple DNA damage responses. The specific initiating event is likely exposure of the otherwise concealed single-stranded 3′ overhang, tandem repeats of TTAGGG, a signal that can be provided to cells in the absence of DNA damage by exogenously provided T-oligos. The ability of T-oligo treatment to trigger SOS-like responses and/or to cause selective apoptosis of already malignantly transformed cells may provide an important new means of cancer prevention and treatment.

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